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LETTER TO THE EDITOR

Differential expression of *SHP-1* in chronic myeloid leukemia

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Despite the unprecedented success of tyrosine kinase inhibitors (TKIs), the clinical management of 20–30% of patients with chronic myeloid leukemia (CML) experiencing primary or secondary resistance to imatinib mesylate (IM) continues to be challenging [1–3]. Early identification of these patients would indicate a more potent agent upfront, or alternative drug following the initial suboptimum response, or stem cell transplant (SCT) prior to the subject becoming refractory to further treatment. Therefore, a biomarker with proven clinical utility of predicting patients' response to IM would assist considerably in optimizing clinical management for such patients. Recently, investigators reported that Src homology 2 domain-containing phosphatase-1 (*SHP-1*) expression levels at diagnosis were prognostic and predictive of TKI response in patients with CML [4]. Previously, others suggested that down-regulation of *SHP-1* contributes to constitutive activation of Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling and disrupts protein phosphatase 2A (PP2A) mediated *BCR-ABL1* elimination, thereby triggering CML transformation [5].

Therefore, we retrospectively studied 97 cDNA samples from patients with highly heterogeneous CML to assess the clinical utility of measuring *SHP-1* mRNA levels in patients with CML (Table I). The samples were collected at various time points, reflected by the overlap in *BCR-ABL1* transcript numbers for those who achieved a major molecular response (MMR) and those who did not (Table I). Of the 97 patients, 24 were had advanced disease (AD), i.e. accelerated phase (AP) $n = 6$ and blast crisis (BC) $n = 18$, and 73 patients were in highly heterogeneous chronic phase (CP) treated with different modalities. For 35 of the 73 patients in CP the MMR status was available for assessing the clinical utility of *SHP-1* levels. Among the 24 patients in AD, at least five archived serial mRNA samples were available for each of the five patients for longitudinal studies. Of these five patients, four had been treated with one or more TKIs and one had undergone allogeneic SCT. We also included a cohort control

of 77 diagnostic samples from a group of patients with heterogeneous acute myeloid leukemia (AML) and 18 normal control samples from adult volunteer blood donors, whose characteristics are detailed in Table I.

SHP-1, *BCR-ABL1* and endogenous control gene, *GUSβ*, transcripts were quantified by real time polymerase chain reaction (Q-PCR) as previously reported [6]. Standard curves were constructed for each assay using serial log dilutions of plasmid, ranging from 1×10^3 to 1×10^6 , with target gene specific insert. *BCR-ABL1* and *GUSβ* target sequences were included in one plasmid and the other included the *SHP-1* insert (a kind gift from Professor F. Pane, Naples, Italy). Only those samples with ≥ 5500 *GUSβ* transcripts were evaluated for this report. Non-parametric Mann-Whitney tests were performed using PRISM software.

Briefly, 38 of the 73 patients in CP were prescribed single agents: (interferon and cytarabine [$n = 1$]), IM ($n = 30$), nilotinib ($n = 6$) or dasatinib ($n = 1$). The remainder were treated with two or more agents, as were the 24 patients with AD. *SHP-1* mRNA was detectable in all samples screened by Q-PCR (Table I). However, a significant differential in mRNA expression ($p < 0.0001$) was observed between patients in CP and the normal control group. Furthermore, the *SHP-1* transcripts were significantly lower ($p = 0.0001$) in patients with AD, with a median of 14.0 (range 0.8–211.9), in comparison to patients in CP, median 35.7 (range 5.2–675.1). Similarly, we observed a significant difference between patients with CML with AD and normal control samples ($p < 0.0001$). However, we observed no significant difference in *SHP-1* levels between AML and normal control samples ($p = 0.801$). This is probably explained by the molecular heterogeneity among the patients with AML, in contrast to the single genetic lesion associated with CML, and that *SHP-1* is reported to bind to *BCR-ABL1*.

In contrast to published data [4] we found no significant difference ($p = 0.0966$) between patients who failed to achieve a MMR within 18 months ($n = 22$) and those

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Table I. Summary of sample groups.

Subjects*	n	Sex, M/F	Age (years) (median)	BCR-ABL/GUSβ (median)	SHP-1/GUSβ (median)
Normal controls	18	8/10	35-61 (44)	—	1.40-6.36 (3.66)
AML	77	42/35	8-85 (63)	—	0.56-13.29 (3.50)
CML: CP	73	44/29	19-75 (63)	0-1053 (18.38)	5.18-675.1 (35.69)
CML: AD†	24	16/8	34-74 (61)	0.40-1947 (182.7)	0.82-211.9 (14.0)
CML: MMR	13	8/5	20-66 (52)	0.24-140.30 (7.30)	15.46-318.9 (35.69)
CML: f-MMR	22	17/5	19-72 (32)	0.0-197.60 (62.33)	6.31-162.1 (26.72)

AML, acute myeloid leukemia; CML, chronic myeloid leukemia; CP, chronic phase; AD, advanced disease; MMR, major molecular response; f-MMR, failed MMR.

*From among the total 97 patients with CML, 13 were classified as having achieved MMR and 22 did not.

†Six accelerated phase, 18 blast crisis.

patients who did ($n = 13$). To exclude the possibility that the statistical value might have been influenced by either the highly variable collection time-points or the diverse therapeutic agents administered, a restricted analysis of 15 patients treated with IM alone and for whom we had samples collected at diagnosis was performed. Even within this group we found no significant difference ($p = 0.4527$), i.e. between those who did ($n = 6$) and failed to ($n = 9$) achieve MMR within 12 months. This did not change even when the criterion was extended to 18 months. This variance from published data may reflect differences in the timing of sample collection during the course of treatment in this study and that reported by Esposito *et al.* [4]. However, these data do not exclude the possibility that assessing SHP-1 activity at the protein level would be predictive. Nevertheless, protein analysis is too complex for a clinical laboratory to perform, in contrast to Q-PCR analysis, and therefore not within the scope of this assessment.

In addition we noted no significant difference in *SHP-1* mRNA levels between those patients in CP who had been prescribed one ($n = 37$), two ($n = 7$) or ≥ 3 TKIs ($n = 8$), which generally correlated with optimal, suboptimal and/or failed response.

The kinetics data were consistent with overall CP and AD results, showing that *SHP-1* levels decreased as the *BCR-ABL1* transcript numbers increased, i.e. an inverse relationship (Figure 1), implying that regulatory control of the two is directly or indirectly linked. We did note that for patient 4, including the period when the subject was in CP (Figure 1), this relationship was not observed. However, there was no difference of note in this patient's clinical history compared to the other four subjects. More importantly, *BCR-ABL1* transcripts in these five patients were not preceded by a decrease in *SHP-1*.

Given the relatively low levels of *SHP-1* in comparison to *BCR-ABL1* expression, we confirmed that our assay could

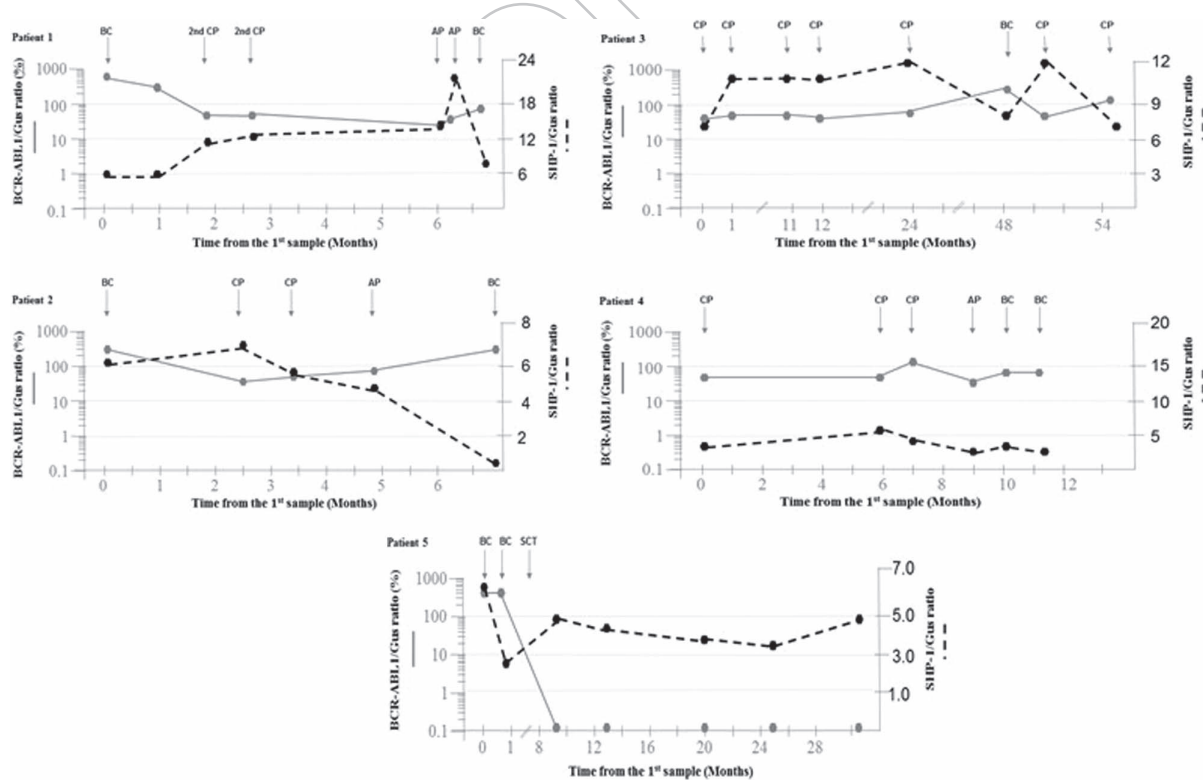


Figure 1. *SHP-1* and *BCR-ABL1* kinetics. Kinetics data for *SHP-1* (dashed lines) and *BCR-ABL1* (solid lines) are shown for five patients with CML included in the longitudinal study. Y-axis for *SHP-1* levels is on the right of each graph. *SHP-1* mRNA was detected in all samples tested for the five patients and reflected the *BCR-ABL1* kinetics. *SHP-1* levels did not predict a change in patients' disease status, such that an increase or decrease in its expression did not precede a change in *BCR-ABL1* transcript levels. Therefore, we conclude that its predictive value is not superior to that of the disease specific marker, *BCR-ABL1*.

reproducibly detect a five-fold change in *SHP-1* mRNA levels by titrating, in duplicate, the SU-DHL-1 cell line with the LAMA-87 hematopoietic cell line. This is consistent with the generally accepted view that Q-PCR assays have a dynamic range of 5-log, although up to an 8-log range is achievable.

Therefore, the kinetics and MMR data suggest that measuring the *SHP-1* mRNA level does not provide additional information for identifying patients at risk of disease progression or predicting response to TKIs beyond that gleaned from close regular monitoring by measurement of disease-specific *BCR-ABL1* transcripts. However, the differential expression of *SHP-1* between CP and AD observed in this study was consistent with earlier reports suggesting that phosphatase antagonizes *BCR-ABL1* ability to block differentiation [7,8]. Reduced expression of *SHP-1* might free *BCR-ABL1* to recruit and activate JAK2. Active JAK2 has been reported to enhance β -catenin activity and inactivate PP2A mediated degradation of *BCR-ABL1*, thus triggering BC [9].

In conclusion, our data imply that *SHP-1* levels fail to predict TKI response. However, in keeping with previous reports, our data provide further evidence to support the notion that *SHP-1* plays a role in CML disease progression.

Potential conflict of interest: Disclosure forms provided by the authors are available with the full text of this article at www.informahealthcare.com/lal.

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